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Polymeric Delivery Systems for Biopharmaceuticals

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Introduction: biopharmaceuticals and polymeric drug delivery

The term *biopharmaceutical* refers to recombinant peptides and proteins that are biologically active and used to prevent or treat diseases. Biopharmaceuticals present one of the most dynamic and promising sectors of the pharmaceutical industry, having enjoyed a rapid expansion over the past few years with compounded growth rates exceeding double-digit figures – largely outpacing the overall performance of the pharmaceutical market. One of the major challenges regarding biopharmaceuticals is their complex chemical structure, which gives rise to stability concerns and requires novel delivery approaches. Polymeric devices have been found to enhance both the stability of biopharmaceuticals and their delivery profile.

During the past three decades, considerable progress has been established in the development of new polymers for the preparation of suitable drug delivery systems. Polymers applied in drug delivery are mainly categorized into two groups; namely, biodegradable and non-biodegradable polymers. Both of these two types of polymers should be safe, non-toxic, and biocompatible in order to be able to be

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Abbreviations: BSA, bovine serum albumin; CFA, complete Freund's adjuvant; CyDs, cyclodextrins; EVAc, ethylene vinyl acetate co-polymer; HPMA, N-(2-hydroxypropyl) methacrylamide; MALTs, mucosal associated lymphoid tissues; MCC, mono-N-carboxymethyl chitosan; Ons, oligonucleotides; PAA, polyacrylic acid; PAGA, poly[alpha-(4-aminobutyl)-L-glycolic acid]; PCL, polycaprolactone; p(DMAEMA), poly(2-(dimethylamino) ethyl methacrylate); PEC, poly(epsilon-caprolactone); PEI, polyethyleneimine; PELA, polylactide-co-poly(ethylene glycol); PEO, poly(ethylene oxide); PEO-cl-PEI, poly(ethylene oxide) and polyethyleneimine; PLA, poly(L-lactide); PLG, poly(D,L-lactide-co-glycolide); PLGA, poly(D,L lactic-co-glycolic acid); PMMA, polymethylmethacrylate; POE, poly(orthoesters); Poly-HEMA, polyhydroxyethylmethacrylate; PPEs, polyphosphoesters; SPH, superporous hydrogel; SPHC, superporous hydrogel composite; TMC, N-trimethyl chitosan chloride.

Biotechnology and Genetic Engineering Reviews – Vol. 21, November 2004
0264–8725/04/21/147–181 $20.00 + $0.00 © Intercept Ltd, 9 Farriers Close, Colehill, Wimborne, Dorset, BH21 2UA, UK

administered to human beings. The source of these polymers can be either natural or synthetic, and either of these two sources might be structured as homo/hetero-polymers or co-polymers. In this review article, the most applicable polymers in drug delivery are considered.

Polymers in drug delivery

BIODEGRADABLE POLYMERS

Biodegradable polymers may be defined as synthetic or natural polymers which are degradable *in vivo*, either enzymatically or non-enzymatically, to produce biocompatible or non-toxic by-products that can be further metabolized or excreted via normal physiological pathways (Jalil, 1990). Heller has defined three mechanisms of polymer erosion (Heller, 1980). *Mechanism I* concerns polymers such as cross-linked gelatin, collagen, or poly(vinyl alcohol), which are hydrophilic and make water insoluble by their hydrolytically unstable cross-links. These polymers are mainly used for the release of sparingly water-soluble drugs, and for macromolecules such as enzymes and antigens (Deshpande *et al.*, 1998).

Mechanism II includes polymers such as poly(vinyl methyl ether/maleic anhydride) that are initially water insoluble, and are solubilized by ionization or protonation of a pendent group without any backbone cleavage. These polymers are therefore not biodegradable, and cannot be used for implants because of the difficulty of their elimination.

Mechanism III refers to hydrophobic polymers such as poly-lactide, poly-glycolide and their co-polymers, that can be converted to small soluble molecules by backbone cleavage. As long as these breakdown products are not toxic, these polymers are suitable as implantable carriers for the administration of drugs to any organ. Most biodegradable polymers, such as polyesters, polyanhydrides, poly(orthoesters), polyamides and their co-polymers, belong to this group of polymers. Their biodegradation can be classified into two erosion patterns: *bulk erosion* and *surface erosion* (Langer and Peppas, 1983). In bulk erosion, the entire area of polymer matrix is subjected to chemical or enzymatic reaction; thus, erosion occurs homogeneously through the entire matrix. Bulk eroding polymers degrade all over their cross-section, have erosion kinetics which are non-linear, and are usually characterized by a discontinuity (Goepferich, 1997). In bulk erosion, the size of a device will remain constant for a considerable portion of time during its application (Langer, 1990). In surface erosion, polymer degradation is limited to the surface of an implant exposed to a reaction medium. Erosion therefore starts at the exposed surface and works downwards, layer by layer. The advantage of surface-eroding polymers is the predictability of the erosion process (Goepferich and Langer, 1995a). Thus, a drug distributed homogeneously in a surface-eroding matrix implant, of which the surface is invariant with time, shows constant release with time over the period of implantation (Goepferich and Langer, 1995b).

Several classes of synthetic biodegradable polymers, including aliphatic polyesters, polyorthoesters, polyanhydrides, polyphosphoesters, polyphosphazene, polyamino acids, polyamides, poly(alkyl cyanoacrylates), poly(carbonates), and poly(imino carbonates), have been proposed for controlled drug delivery. Among

these, the first three groups have generated immense interest because of their excellent biocompatibility and biodegradability, in addition to the possibility of synthesis of different types of these polymers with various physicochemical characteristics for different medical and pharmaceutical applications.

Polyesters

The linear polyesters are by far the most widely studied class of biodegradable polymers. Poly(glycolic acid) was the first synthetic polymer used to prepare bioabsorbable sutures (Schmitt and Polistina, 1967; Frazza and Schmitt, 1971; Brady *et al.*, 1973). The first such application of a biodegradable matrix in controlled drug delivery was reported by Yolles in 1970 and involved the release of narcotic antagonists from lactic acid polyesters (Yolles *et al.*, 1970, 1973). The principal polymers of this class are homo- and co-polymers derived from lactic and glycolic acid and its co-polymers (*Figure 6.1a*). These polymers are synthesized by the ring opening melt condensation of the cyclic dimers, lactide and glycolide (Dittrich and Schulz, 1971; Kulkarni *et al.*, 1971). Due to the asymmetrical carbon of lactic acid, D and L stereoisomers exist, and the resulting polymer can be D, L, or racemic DL which can have different physicochemical properties.

A well-proven advantage of the lactide/glycolide polymers is the versatility in polymer properties and performance characteristics. For wide applications in controlled drug delivery, it is imperative that a range of rates and duration of drug release be achievable. A broad spectrum of performance characteristics can be reached by a careful manipulation of four key variables, including monomer stereochemistry, co-monomer ratio, polymer chain linearity, and polymer molecular weight (Lewis, 1990). Poly(lactic acid) (PLA) is more hydrophobic than poly(glycolic acid) (PGA), which is provided by the extra methyl group. Polylactide-glycolide (PLGA) polymers can be prepared in different molar ratios of lactic to glycolic acids, as the proportion is critical in determining *in vivo* degradation rate (Lewis, 1990). PLGA, PLA and their co-polymers have been used extensively for the preparation of drug delivery systems. Recently, we have used these polymers for the controlled delivery of levonorgestrel as a contraceptive agent and naltrexon as an opioid antagonist (Dinarvand *et al.*, 2001, 2003).

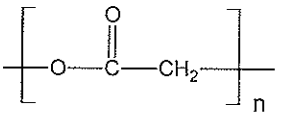
Another polymer from the polyester family which has been evaluated for use as a long-term delivery system is polycaprolactone (PCL). The propensity of ε-caprolactone to undergo ring-opening polymerization was first established by Carruthers in his classic studies of polyesters during the early 1930s (van Natta *et al.*, 1934). This polymer was used, for example, in 1973 as a subdermal delivery system for contraceptive steroid under the trademark Capronor.

Compared to PLA and PLGA, PCL is semicrystalline, rather hydrophobic, and has a high molecular weight. The crystallinity of PCL varies with its molecular weight (Pitt *et al.*, 1981), in which for a molecular weight of ~100 000 Da the crystallinity is about 40%, rising to 80% as the molecular weight decreases to 5000 Da. Since the crystalline phase is inaccessible to water and other permeants, an increase in crystallinity reduces the permeability by both reducing the solute solubility and increasing the difficulty of the diffusional pathway (Pitt, 1990).

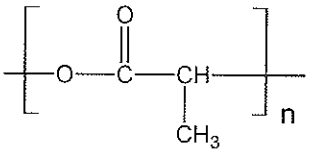
As with PLA and PLGA, PCL is hydrolysed by random chain scission through

(a) polyesters

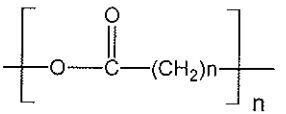
1-poly(glycolic acid)



2-poly (lactic acid)

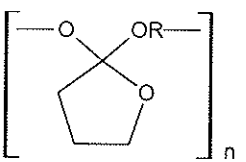


3-polycaprolactone

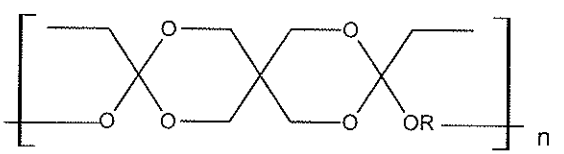


(b) polyorthoesters

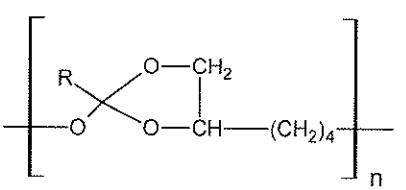
1-POE I



2-POE II



3-POE III



4-POE IV

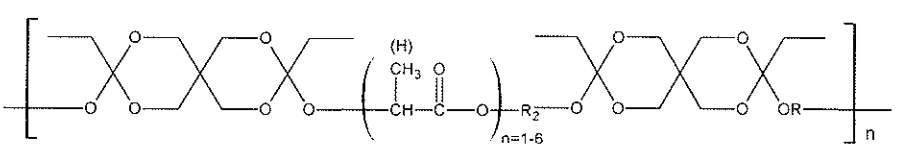


Figure 6.1. Chemical structure of polyesters and polyorthoesters.

hydrolytic cleavage of ester groups to ε-hydroxycaproic acid, leading to a continual decrease of the molecular weight without any significant loss of the device weight. Enzyme catalysis is excluded in this first phase, because the diffusion of such high molecular weight substances is impossible in the polymer bulk. When the molecular weight reaches about 5000 Da, the cleavage of the chain is catalysed by enzymes and accompanied by a loss of weight resulting from the diffusion of small polymeric fragments from the matrix; these fragments are subsequently removed by phagocytosis (Pitt and Schindler, 1984; Einmahl *et al.*, 1999).

Poly(orthoesters)

Poly(orthoesters) (POE) have been under development since 1970, and during this period, four polymer families have been developed (*Figure 6.1b*). POE I, the first such polymer prepared, has been developed at Alza Corporation and described in a series of patents by Choi and Heller (1978, 1979). Solid POE I has been used in the treatment of burns (Vistnes *et al.*, 1976), in the delivery of the narcotic antagonist naltexone (Capozza *et al.*, 1978), and in the delivery of the contraceptive steroid levonorgestrel (Benagiano *et al.*, 1979). These polymers have been investigated also by Sudmann's group in a number of orthopaedic applications (Pinholt *et al.*, 1992; Sudmann *et al.*, 1993). However, all work with this polymer has now been discontinued. The main reason for this is the lack of control over polymer erosion due to the autocatalytic nature of hydrolysis (Heller *et al.*, 2000).

The second generation of POEs (known as POE II) was developed at the Stanford Research Institute (Heller, 1990). When POE IIs are hydrolysed, they give (at least initially) neutral products, so that it is not necessary to use bases to neutralize products from acidic hydrolysis (Einmahl *et al.*, 2001). POE IIs are also extremely hydrophobic, and thus very stable. Therefore, in order to achieve shortened erosion times, it is necessary to use small amounts of acidic excipient, such as suberic acid, that are physically incorporated into the polymer (Sparer *et al.*, 1984). This family has been tested in numerous applications, such as contraceptive agents (Heller *et al.*, 1981, 1985a,b), anticancer drugs (Heller *et al.*, 1987; Seymour *et al.*, 1994), chemotherapic agents (Vistnes *et al.*, 1976; Du *et al.*, 1997), and orthopaedic applications (Daniels *et al.*, 1990; Andriano *et al.*, 1999; Solheim *et al.*, 2000).

The third generation of POEs, POE III, is a viscous polymer that was developed at the University of Geneva in the 1990s (Merkli *et al.*, 1993). This generation is characterized by an ointment-like consistency, providing significant and unique advantages, such as the ability to incorporate therapeutic agents or additives by simple mixing, without the need to use solvents or elevated temperatures, which allows fragile and thermolabile drugs – peptides, proteins, or oligonucleotides – to be formulated (Einmahl *et al.*, 2001). Furthermore, the polymer is easily injected using a conventional syringe with an appropriate needle, which is an improvement when compared to solid devices that must be placed either with a trocar or through a more complex surgical procedure.

POE IV is a modification of POE II that allows control over erosion rates without the need to add acidic excipients. One of the serious problems with POE II is the diffusion of the acidic excipient from the polymer that not only complicates kinetics of drug release, but more importantly, eventually leads to an excipient-depleted

polymer which remains in the tissue for a significant time (Heller and Gurny, 1999). One good approach is to incorporate into the polymer backbone a short segment that readily hydrolyses to an acidic product that acts as the acidic excipient and catalyses the hydrolysis of orthoester linkages in the polymer (Heller and Gurny, 1999). Then, by controlling the concentration of such segments in the polymer, the rate of erosion can be accurately controlled without complications arising from excipient diffusion (Heller *et al.*, 2002). Such polymers have been designated as *autocatalysed poly(orthoesters)* (POE IV). These polymers are viscous and injectable, depending on polymer structure (Ng *et al.*, 1997).

POE III and IV undergo surface erosion and heterogeneous degradation, which allows concomitant drug release, following zero-order kinetics without initial or final release. Erosion and release rate can be modulated by many parameters, such as polymer molecular weight and structure (for POE IV), as well as physicochemical properties of incorporated additives or drugs (Schwach-Abdellaoui *et al.*, 2001).

Polyanhydrides

Polyanhydrides were developed by the textile industry because of their fibre-forming properties (Bucher and Slade, 1909), but interest waned as it was determined that their hydrolytic lability prevented their application for such purposes (Conix, 1958).

In the 1980s, Langer was the first to exploit the hydrolytically unstable nature of polyanhydrides for sustained release of drugs in controlled drug delivery applications (Langer and Peppas, 1983; Rosen *et al.*, 1983; Leong *et al.*, 1985; Mathiowitz *et al.*, 1988; Langer, 1990).

There is a substantial variability regarding the design of polyanhydrides. They can be manufactured as aliphatic or aromatic homopolymers and co-polymers, as well as cross-linked or branched polymers (*Figure 6.2a*). Aliphatic polyanhydride homopolymers are often problematic materials as they are usually highly crystalline with unfavourable mechanical properties (Goepferich and Tessmar, 2002). Aromatic polyanhydrides erode slower than aliphatic ones (Leong *et al.*, 1985), which is due to their increased hydrophobicity and the hindered approach of water to the anhydride bond (Tamada and Langer, 1992).

Polyanhydrides have been investigated as a candidate for controlled release device for drugs used to treat eye disorders (Albertsson *et al.*, 1996), chemotherapeutic agents (Park *et al.*, 1998; Stephens *et al.*, 2000), neuro-active drugs (Kubek *et al.*, 1998), anticancer agents (Domb and Ringel, 1994; Olivi *et al.*, 1996), and macromolecules, such as hormones and enzymes (Thomas *et al.*, 1997).

Although many of the applications already mentioned require the use of polyanhydrides because of their quick erosion, this also coincides with the goal of short-term drug delivery. An example is the development of pulsatile drug delivery systems. Polyanhydride matrices undergo degradation by surface erosion because the rate of polymer hydrolysis is relatively rapid, and mass is lost more rapidly from the surface than from the bulk. The rate of erosion and subsequent drug release is more predictable from surface-eroding than from bulk-eroding matrices (Goepferich, 1997). Advantage can be taken of this property in the development of pulsatile delivery systems. If a polyanhydride matrix implant has been made of several layers

of polymer carrying different doses of the same drug or different drugs, the drugs should be releasable one after another in a pulsatile manner. This principle could be used for a number of drug delivery applications, such as vaccination or local tumour therapy (Wuthrich *et al.*, 1992).

Polyphosphoesters

Biodegradable polyphosphoesters (PPEs) have been investigated since the 1980s as biomaterials, initially in drug delivery, and more recently in gene delivery and tissue engineering (Zhao *et al.*, 2003). Depending on the nature of the side chain connected to the phosphorus, these polymers are conventionally called polyphosphates (P-O-C), polyphosphonates (P-C), or polyphosphites (P-H) (*Figure 6.2b*).

Aside from degradability, this class of polymer is attractive because of its versatility. Manipulation of either the backbone or the side chain structure affords an expansive variety of physicochemical properties. The pentavalency of the phosphorus atom allows for the chemical attachment of drug molecules to the polymer for a biodegradable pendent delivery system (Brosse *et al.*, 1989). Water-insoluble PPEs have been investigated as biodegradable and biocompatible polymers with potential applications as a drug delivery vehicle for low molecular drugs, proteins, DNA plasmid, and tissue engineering scaffolds (Dahiyat *et al.*, 1995; Wan *et al.*, 2001; Wang *et al.*, 2001; Xu *et al.*, 2002).

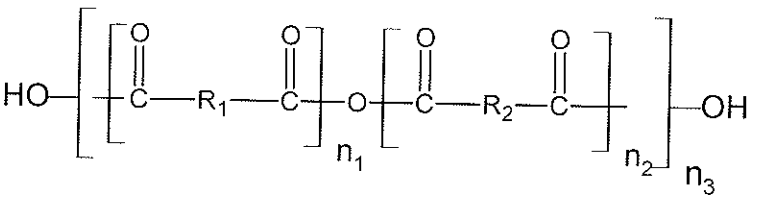
A new class of hydrophobic PPE is a co-polymer of PPE and poly(D,L-lactide). The degradation rate of a poly(lactide-co-phosphate) is mainly controlled by the percentage of phosphate components introduced into the backbone (Chaubal *et al.*, 2003). This has the effect of eliminating the biphasic degradation behaviour typically exhibited by the crystalline PLA. The higher the phosphate contents in the backbone, the faster the degradation rate of the polymers (Mao *et al.*, 1999).

Water soluble cationic polymers are potentially useful for gene delivery (de Smedt *et al.*, 2000; Pouton and Seymour, 2001). In the water-soluble form, the cationic PPEs comprise a new family of gene carrier. Electrostatic interaction between the cationic polymers and negative DNA molecules results in the formation of complexes or nanoparticles, providing protection to DNA from enzyme degradation, and facilitating the cellular uptake of the DNA. Recently, biodegradable PPEs, including polyphosphate and polyphosphoramidates, have been developed as gene carriers (Wang *et al.*, 2001, 2002).

Polyphosphazenes

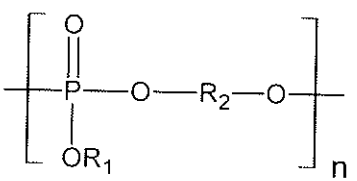
Poly(phosphazenes) are a novel class of high molecular weight polymers consisting of a long chain backbone of alternating phosphorus and nitrogen atoms, with two side groups attached to each phosphorus (Allcock *et al.*, 1988). Most of the poly(phosphazenes) synthesized are hydrolytically stable. But the versatile fact about poly(phosphazene) chemistry is that the phosphorus–nitrogen backbone can be rendered hydrolytically unstable by substituting with appropriate side groups (Ibim *et al.*, 1996). The degradation products of these polymers are usually nontoxic, such as phosphates, ammonia, and side chain groups (White and Singler,

(a) poly anhydride

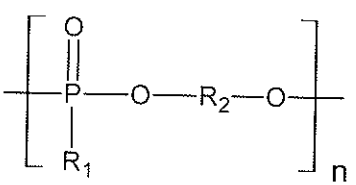


(b) polyphosphoesters

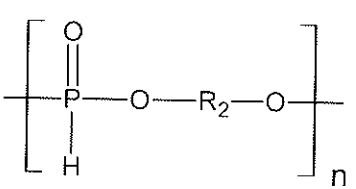
1-polyphosphates



2-polyphosphonate

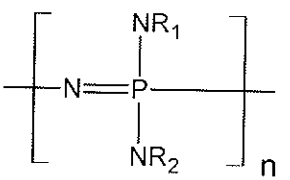


3-polyphosphite



(c) polyphosphazene

1-aminated polyphosphazene



2-alkoxy substituted polyphosphazene

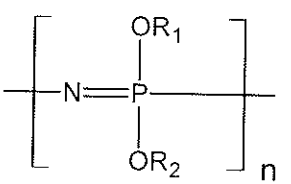


Figure 6.2. Chemical structure of polyanhydrides, polyphosphoesters and polyphosphazenes.

1975; Goedemoed *et al.*, 1991; Allcock, 1992). Biodegradable poly(phosphazenes) can be broadly classified into two groups, depending on the type of side group substituents: those substituted with amines of low pKa (aminated poly(phosphazenes)), and those substituted with activated alcohols (alkoxy substituted) (*Figure 6.2c*) (Scopelianos, 1994).

Poly(phosphazenes) can be degraded by both surface and bulk erosion (Laurencin *et al.*, 1992). The rate of degradation depends on several factors, such as the type and ratio of side group substitute, lability of the bond, ease of water permeability to the polymer matrix, which in turn depends on the hydrophilicity/hydrophobicity of the matrix, solubility of the degradation products, pH, and temperature of the environment (Lakshmi *et al.*, 2003).

Most studies using biodegradable poly(phosphazenes) have used these polymers as pellets or films as drug delivery matrices. These pellets or films are usually fabricated by compression moulding (Crommen *et al.*, 1992) or solvent casting, since they are soluble in a wide range of solvents (Laurencin *et al.*, 1987; Ibim *et al.*, 1996). In addition, pharmaceutical agents or peptides can be linked to poly(phosphazene) backbone (Allcock and Fuller, 1980; Grolleman *et al.*, 1986). *In vivo* hydrolysis would release the active agent in a controlled manner.

NON-BIODEGRADABLE POLYMERS

In contrast to the compounds described as biodegradable polymers, the non-biodegradable group of polymers cannot be degraded *in vivo* by hydrolytic and/or enzymatic means. As a result, these macromolecules need retrieval or further manipulation after introduction into the body, which can be a potential drawback of these polymers in drug delivery. Nevertheless, these polymers offer some favourable properties, including release-controlling and/or supportive barrier film forming, stimuli-responsiveness, bioadhesion, ideal sol–gel transition, polymeric matrix formation, ideal particulate carrier forming, and the capability of complexation with a variety of drugs, biopharmaceuticals, tissue identifiers, and spacers, which, taken as a whole, make them noteworthy in drug delivery research.

The majority of non-biodegradable polymers are chemically based on a C–C backbone (Uhrich *et al.*, 1999), with the representative chemical classes used for drug delivery purposes including polypropylene (PP) (Junginger *et al.*, 1989; Streubel *et al.*, 2002), polyvinyl alcohol (PVA) (Davies *et al.*, 1991; Mumper *et al.*, 1996; Peppas and Mongia, 1997; Li *et al.*, 1998a; Wang *et al.*, 1999; Orienti *et al.*, 2000), polyethylene vinyl acetate (EVAc) (Seki *et al.*, 1991; Katre, 1993), polycarbophil (Clausen and Bernkop-Schnurch, 2001; Cuna *et al.*, 2001), polyacrylic acid (PAA) derivatives (Langer *et al.*, 1997; Peppas and Wright, 1998; Dickinson *et al.*, 2001), polymethacrylates (e.g. polymethylmethacrylate, PMMA, and polyhydroxyethylmethacrylate, poly-HEMA) (Orienti *et al.*, 1992; Langer *et al.*, 1997; Ahlin *et al.*, 2002), polyacrylamides (e.g. N-isopropylacrylamide) (Tomer and Florence, 1993; Shah *et al.*, 1997; Soppimath *et al.*, 2001; Jeong *et al.*, 2002), polymethacrylamides (e.g. N-(2-hydroxypropyl) methacrylamide, HPMA) (Kopecek *et al.*, 1991; Oupicky *et al.*, 2000; Kasuya *et al.*, 2001; Seymour *et al.*, 2002; Stastny *et al.*, 2002), acrylic resins (Eudragits) (Dutta *et al.*, 1995; Krogars *et al.*, 2000; Rafiee-Tehrani *et al.*, 2001), silicones (Diaz *et al.*, 1982; Li

et al., 1998b; Kajihara *et al.*, 2001), and cellulose derivatives (Khan *et al.*, 2000). These polymers have been used successfully in bioadhesives (Peppas and Mongia, 1997; Clausen and Bernkop-Schnurch, 2001; Cuna *et al.*, 2001), pH- (Orienti *et al.*, 2000; Soppimath *et al.*, 2001), thermo- (Jeong *et al.*, 2002), magnetic- (Dutta *et al.*, 1995) and photo-responsive (Tomer and Florence, 1993) delivery systems, particulate carriers (Orienti *et al.*, 1992; Langer *et al.*, 1997; Wang *et al.*, 1999; Dickinson *et al.*, 2001; Ahlin *et al.*, 2002), drug–polymer conjugates (Katre, 1993; Shah *et al.*, 1997; Kasuya *et al.*, 2001), oral controlled-release systems (Junginger *et al.*, 1989; Khan *et al.*, 2000; Streubel *et al.*, 2002), site-specific delivery systems (Khan *et al.*, 2000; Seymour *et al.*, 2002), implantable devices (Diaz *et al.*, 1982), transdermal/topical (Seki *et al.*, 1991; Kajihara *et al.*, 2001; Rafiee-Tehrani *et al.*, 2001), and ophthalmic (Davies *et al.*, 1991; Langer *et al.*, 1997) delivery systems, hydrogels (Tomer and Florence, 1993; Peppas and Wright, 1998; Stastny *et al.*, 2002), gene delivery (Mumper *et al.*, 1996; Oupicky *et al.*, 2000), and vaccine delivery (Singh and O'Hagan, 1998).

Polymers in biopharmaceutical delivery

POLYMERS FOR PEPTIDE AND PROTEIN DELIVERY

During the past two decades, the revolutionary expansion of methods in biotechnology has facilitated the availability of therapeutic peptides for the rational treatment of chronic diseases. However, the development of suitable dosage forms for optimal therapeutic treatment avoiding the parenteral route could not keep pace with the easy accessibility of those therapeutic agents. Various polymeric devices have been developed for the delivery of peptides either via parenteral or non-parenteral routes (Marschutz and Bernkop-Schnurch, 2000). To gain a sufficient bioavailability of these therapeutic agents, various barriers, including the mucus-layer barrier, the enzymatic barrier, and the membrane barrier, have to be overcome. A promising strategy for achieving this goal is the use of multifunctional polymeric matrices. These matrices are based on polymers that display mucoadhesive properties, a permeation-enhancing effect, enzyme-inhibiting properties, and/or a high buffering capacity, which enhance the stability of these hydrophilic macromolecular compounds (Davis, 1992; Fix, 1996).

The potential of polymers which respond to environmental stimuli, such as temperature (d'Emanuele and Dinarvand, 1995; Dinarvand and d'Emanuele, 1995; Dinarvand *et al.*, 1995), pH, etc., should be considered as a tool for the delivery of proteins and peptides. Using these polymers, it is possible to control the release of peptides in a pulsatile or retarded mode (Sakuma *et al.*, 2001). Dinarvand and d'Emanuele (1995) have described a temperature controlled valve system that releases BSA in response to increase in temperature to above the phase-transition temperature of the hydrogel used as the valve of an impermeable polymeric cylinder. Different routes for administration have been considered for peptide delivery, such as buccal (Bird *et al.*, 2001), oral (Sinko *et al.*, 1999; Bernkop-Schnurch, 2000), or parenteral (Sanders *et al.*, 1991) pathways. For each of these routes, various polymers have been used to develop appropriate delivery systems, and these will now be considered.

Poly(D,L-lactide-co-glycolide) (PLGA)

The application of poly(D,L-lactide-co-glycolide) in peptide and protein delivery has been considered due to the fact that constant release kinetics can be achieved over a period of some days to months (Burton *et al.*, 2000). PLGA microspheres are able to control the release of peptides by combining PLGA polymers which vary in their molecular weights in various ratios. Increasing the component of lower molecular weight 50:50 hydrophilic PLGA polymer (8.6 kDa) may facilitate an increase in the initial release of the drug. This increased initial release of the peptide may avoid the therapeutic lag phase usually observed with microencapsulated macromolecules (Ravivarapu *et al.*, 2000).

Chitosan and its derivatives

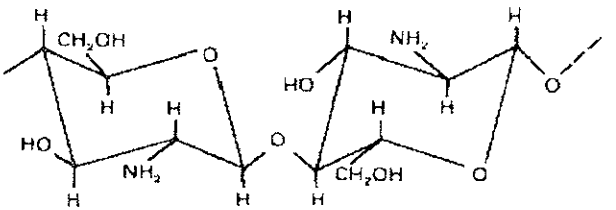
In the past decade, chitosan has been put forward as a suitable compound in various pharmaceutical formulations for the delivery of hydrophilic macromolecules, including peptides and proteins (*Figure 6.3a*). By exchanging the primary amino group with different functional groups at the 2-position of this poly(β1-4D-glucosamine), the features of chitosan can be optimized according to a desired purpose in drug delivery systems. Various derivatives and complexes of chitosan have been developed so far for the delivery of peptides and proteins. Such derivatives include N-trimethyl chitosan chloride (TMC), mono-N-carboxymethyl chitosan (MCC), and chitosan–EDTA conjugates. TMC and MCC are new derivatives of chitosan which are able to open the tight junctions of epithelia and enhance the paracellular permeability of hydrophilic macromolecules, including peptides and proteins (Kotze *et al.*, 1999; Thanou *et al.*, 2001a). These chitosan derivatives interact with junctional proteins, including claudin-1 and occludin, and cause opening of tight junctions by triggering a physicochemical interaction of TMC and MCC with these junctional proteins. It is known that TMC binds to the epithelial cell membrane by electrostatic interaction, resulting in F-actin depolymerization and disbandment of the tight junction protein ZO-1, and finally opening of tight junctions (Banan *et al.*, 2001).

One promising derivative of chitosan is the chitosan–EDTA conjugate, which exhibits the lowest amount of remaining free amino groups, and seems to be a useful tool in overcoming the enzymatic barrier for perorally administered therapeutic peptides (Bernkop-Schnurch and Krajicek, 1998). Covalent attachment of thiol moieties on polyacrylates, cellulose derivatives, and chitosan leads to improved mucoadhesive and permeation-enhancing properties of peptide (Bernkop-Schnurch and Walker, 2001). Chitosan–alginate beads have been shown to offer potential for the oral absorption of insulin by enhancing the stability of peptide against enzymatic degradation (Onal and Zihnioglu, 2002). These derivatives of chitosan have apparently been shown to be non-toxic and biocompatible, which enhances the potential of their application in peptide drug delivery.

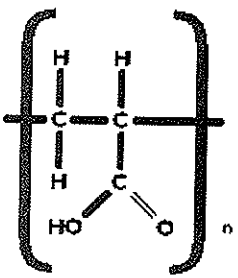
Mucoadhesive polymers

Mucoadhesive polymers are mainly used for drug targeting due to their adhesion properties. Bioadhesion properties of mucoadhesive polymers, such as carbomers

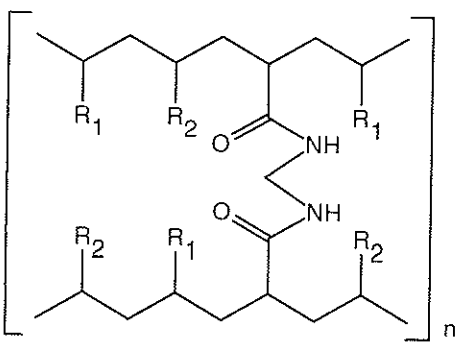
(a) chitosan



(b) carbomer



(c) super porous hydrogel



SPH: $R_1 = CONH_2$, $R_2 = COOH$

SPH composite: $R_1 = CONH_2$, $R_2 = COO(CH_2)_3SO_3K$

Figure 6.3. Chemical structure of chitosan, carbomer and super porous hydrogels.

(*Figure 6.3b*), are an essential factor for improving the bioavailability of peptides and proteins. The development of polymers which are able to stick to various mucosal surfaces of the human is an attractive issue for peptide drug delivery for the

following reasons: the control and delay of peptide release, targeting interaction with the mucus layer at the site of absorption, and enhancing the penetration of peptide across the mucus gel layer and passage through the surface structures of epithelial cells (Lehr *et al.*, 1992). The phenomenon of mucoadhesion is a natural complex involving both kinetic and thermodynamic aspects. The mechanism of bioadhesion of mucoadhesive polymers can be classified into chemical (including electronic and adsorption theories) and physical types (including interfacial phenomena, such as wetting or spreading, and interpenetration or diffusion of polymer chains across the interface) (Peppas and Buri, 1985). Mucoadhesive polymers that have been considered thus far probably have inadequate properties to achieve the goal of peptide drug delivery. It may be necessary to modify these polymers in such a way as to increase their specific absorption properties by incorporating receptor-mediating binding groups in their chemical structures (Thanou *et al.*, 2001b). These polymers are also able to chelate Ca^{+2} by their carboxylic groups, and diminish the extracellular Ca^{+2} concentrations, which results in opening of the tight junctions (Luessen *et al.*, 1995).

Superporous hydrogels

A variety of hydrogels has been employed for different applications in the delivery of peptides and proteins. Due to their biocompatibility and biodegradability, they have been used, for example, as bio-absorbable materials in the delivery of medicines. They are unique carriers for controlled drug delivery, and release can be governed by both the swelling and biodegrading properties of hydrogels (Dorkoosh *et al.*, 2000) (*Figure 6.3c*). New generations of hydrogels are superporous hydrogel (SPH) and SPH composite (SPHC) polymers, which are able to swell very rapidly

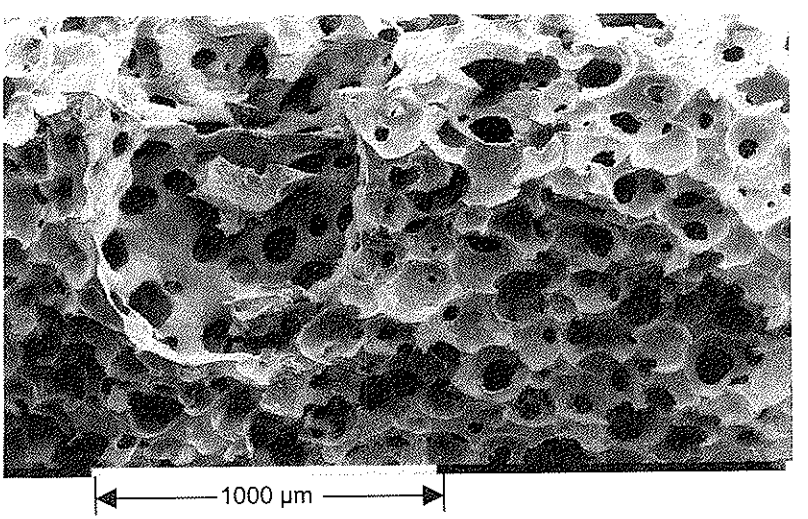


Figure 6.4. Scanning electron microscopy pictures of SPH polymer (copy from Dorkoosh *et al.*, 2000).

compared to conventional hydrogels, which swell slowly due to their rigid crystalline structure and low elasticity in their polymer chains.

The fast swelling properties of these polymers can be related to the capillary wetting of interconnected open pores, forming a large number of interconnected pores (*Figure 6.4*), which causes a rapid absorption of water by the capillary attraction forces within the pores: these polymers therefore swell to their maximum volume very quickly (Dorkoosh *et al.*, 2001).

Novel delivery systems have been developed and characterized using SPH and SPHC polymers for peroral absorption of peptides and proteins. These designed systems are introducing novel approaches with new mechanisms of action for intestinal absorption of peptide and protein drugs. The major advantages of such systems for peptide drug delivery are not only that they can take into account all the necessary factors for oral absorption of peptide drugs, such as inactivation of luminal enzymes, drug targeting, and opening of the tight junctions (Dorkoosh *et al.*, 2002b), but also their capability for achieving a so-called 'time-controlled release profile'. As shown in *Figure 6.5a*, for a normal release profile from the dosage form, drug release starts at 'time zero' (i.e. with no lag), indicating that from the moment the dosage form is in the intestinal lumen, drug release commences. However, for delivery of peptide drugs, a lag time of 20–30 min is necessary to inactivate proteolytic enzymes and to open the tight junctions. Thereafter, a 'burst release' is required in which the whole amount of peptide drug should be released from the dosage form in a short period of time. This type of drug release is depicted in *Figure 6.5b*, and is called a time-controlled release profile (Dorkoosh *et al.*, 2002c).

Furthermore, using these delivery systems, a new mechanism of action for drug targeting has been developed, keeping the dosage form mechanically fixed for a specific period of time at the intestinal site of drug absorption (Dorkoosh *et al.*, 2002a). Delivery systems based on SPH polymers have demonstrated their potential for oral absorption of peptides, insulin, and octreotide. Oral absorption of insulin using an SPH-based delivery system was enhanced at least 2-fold, compared to the control, where no polymers were used (Dorkoosh *et al.*, 2002d). The oral absorption of octreotide using SPH-based delivery systems was shown to increase by up to 16%, compared with the absorption from oral solutions of octreotide (Dorkoosh *et al.*, 2002e). Therefore, SPH and SPHC polymers show potential properties for oral delivery of peptides and proteins.

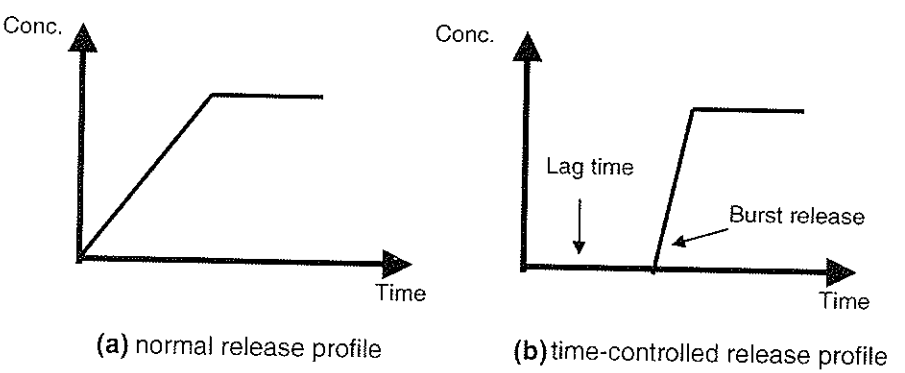


Figure 6.5. Kinetics of drug release from intestinal formulations.

POLYMERS FOR GENE DELIVERY

Advancing biotechnology is spurring the development of new, pharmaceutically engineered gene delivery vehicles. Gene transfer to humans requires carriers for plasmid DNA, which can efficiently and safely carry the gene into the nucleus of the desired cells. A series of chemically different cationic polymers is currently being investigated for these purposes. Although many cationic polymers do indeed condense DNA spontaneously, which is a requirement for gene transfer in most types of cells, the physicochemical and biopharmaceutical behaviour of the current generation of 'polyplexes' severely limits an efficient gene transfer *in vitro*, and especially *in vivo*. However, cationic polymer/DNA complexes are widely used for gene delivery, although the influence of the cationic polymer on the biophysical properties of the resulting complex is poorly understood.

Arigita and co-workers demonstrated that plasmid DNA complexed with either poly(L-lysine) or p(DMAEMA) is protected against digestion by DNase I. They also showed that the differences in transfection potential of the polyplexes cannot be ascribed to differences in binding characteristics, but are probably caused by other factors. As compared with the other polymers they studied, p(TMAEMA) appears to have a high affinity for DNA, as was concluded from the observation that poly(aspartic acid) was unable to fully dissociate complexes containing this polymer (Arigita *et al.*, 1999)

Wolfert and co-workers have used several series of cationic polymers to evaluate the influence of structural parameters on the properties of DNA complexes. The parameters they studied included the length of side chain, charge type (primary versus tertiary and quaternary), polymer molecular weight, and charge spacing along the polymer backbone. They showed that cationic polymers with short side chains (such as polyvinylamine) formed small complexes, resistant to destabilization by polyanions, with low surface charge, limited transfection activity, and efficient intranuclear transcription. Conversely, cationic polymers with long side chains (e.g. poly[methacryloyl-Gly-Gly-NH-(CH_2)(6)-NH_2)] showed inefficient complex formation, high positive surface charge, and better transfection activity. The effects of molecular weight varied between polymers; for example, low molecular weight poly(L-lysine) produced relatively small complexes, whereas low molecular weight poly[2-(trimethylammonio)ethyl methacrylate chloride] produced large aggregates. Polymers containing quaternary ammonium groups showed efficient complex formation, but poor transfection. Finally, spreading charges widely on the polymer structure inhibited their ability to condense DNA. In summary, to achieve small, stable complexes, the use of cationic polymers with short side chains bearing primary amino groups is suggested (Wolfert *et al.*, 1999).

Transgene expression and tumour regression after direct injection of plasmid DNA encoding cytokine genes, such as mIL-12 and mIFN-gamma, remain very low. Maheshwari and co-workers developed non-toxic, biodegradable, polymer-based cytokine gene delivery systems, which should enhance mIL-12 expression, increasing the likelihood of complete tumour elimination. They synthesized poly[alpha-(4-aminobutyl)-L-glycolic acid] (PACA), a biodegradable, non-toxic polymer, by melting condensation. Plasmids used in their study encoded luciferase (pLuc) and murine interleukin-12 (pmIL-12) genes. PAGA/plasmid complexes were

prepared at different (+/-) charge ratios, and characterized in terms of particle size, zeta potential, osmolality, surface morphology, and cytotoxicity. Polyplexes prepared by complexing PAGA with pmIL-12, as well as pLuc, were used for transfection into cultured CT-26 colon adenocarcinoma cells, as well as into CT-26 tumour-bearing BALB/c mice. These researchers showed that the PAGA/pmIL-12 complexes did not induce any cytotoxicity in CT-26 cells, as evidenced by 3-{4,5-dimethylthiazol-2-yl}-2,5-diphenyltetrazolium bromide assay, and revealed enhanced anti-tumour activity *in vivo*, compared to naked pmIL-12. PACA/pmIL-12 complexes were non-toxic and significantly enhanced mIL-12 expression at mRNA and protein levels, both *in vitro* and *in vivo* (Maheshwari *et al.*, 2000).

Kono and co-workers have described that complexation of the pH-sensitive, fusigenic liposome with a lipoplex consisting of 3 beta-(N-(N',N'-dimethylaminoethane) carbamoyl)cholesterol, dioleoylphosphatidylethanolamine and plasmid DNA gives efficient gene delivery systems. They prepared the complexes, which are termed SucPG-complexes, with a positively or negatively charged surface by mixing the lipoplex with varying amounts of the SucPG-modified liposomes. The positively charged SucPG-complexes, either bearing or not bearing a cell-specific ligand, transferrin, could transfect HeLa cells efficiently. In contrast, the negatively charged complexes hardly transfected the cells when transferrin was not conjugated with them. However, the negatively charged SucPG complexes bearing transferrin exhibited high transfection ability against HeLa and K562 cells, indicating that this gene delivery was achieved through their binding to the cellular receptors. These transferrin-attached, negatively charged complexes retained high transfection ability in the presence of serum. Thus, this negatively charged complex may be useful as a non-viral vector *in vivo* (Kono *et al.*, 2001).

Protein expression after delivery of plasmid DNA to the cell nucleus depends on the processes of transcription and translation. Cytotoxic gene-delivery systems may compromise these processes and limit protein expression. This situation is perhaps most prevalent in current non-viral, polycationic gene-delivery systems in which the polycationic nature of the delivery system can lead to cytotoxicity. To approach the problem of creating non-toxic but effective gene-delivery systems, it has been hypothesized that, by optimizing the balance between polymer cationic density with endosomal escape moieties, effective gene transfer with low cytotoxicity could be created. As a model system, a series of polymers has been synthesized whose side chain termini vary with respect to the balance of cationic centres and endosomal escape moieties (Putnam *et al.*, 2001).

Hennink and co-workers have found out that poly(2-(dimethylamino) ethyl methacrylate), p(DMAEMA), a cationic acid and water-soluble polymer, is able to condense plasmid DNA by electrostatic interactions. The ability of the resultant polyplexes to transfect cells greatly depends on their characteristics: small (<0.15 μm), with positively charged particles showing the highest transfectivity. The polyplexes preserved almost their full transfection potential after aging for 10 months at 4 and 20°C, but not at 40°C. After storage, conformational changes in the secondary and tertiary structure of DNA were observed by these researchers. Freeze-dried polyplexes, with sucrose as lyoprotectant, almost fully retained their transfection efficiency, even when aged for 10 months at 40°C (Hennink *et al.*, 2001).

POLYMERS FOR VACCINE DELIVERY

Vaccines are antigenic biopharmaceuticals capable of inducing the humoral and/or cell-mediated immunity in the host organism against the desired pathogen micro-organisms following their administration via a procedure named *vaccination*. An ideal delivery vehicle for vaccine delivery should be safe (i.e. non-toxic as well as non-immunogenic), economical, effective and reproducible at inducing the proper immune responses to the delivered antigen, pharmaceutically acceptable (i.e. stable and biocompatible), and, finally, allow non-invasive vaccination with the possibility of increasing compliance. In addition, an optimal vaccine delivery system should deliver the antigens in a manner that mimics the natural infection, in which there is a large initial load of antigens delivered from the infected cells, followed by a decreasing amount of antigens, and possible for a period of several weeks (Ada, 1991).

The problems associated with the development of controlled-release vaccines may be greater than those for therapeutic proteins, since the required duration of release is likely to be much longer (O'Hagan *et al.*, 1998). Polymeric controlled-delivery systems can potentially deliver either the antigen or adjuvants to the desired location at predetermined rates and durations to generate an optimal immune response. The carrier may also partially protect the vaccine from degradation until it is released. The combination of slow release and tissue depot effect may reduce the amount of antigens or adjuvants used in the vaccine, and eliminate the booster shots that are necessary for the success of many vaccinations. Moreover, the adjuvant effect of the polymeric delivery systems is another remarkable advantage of these carriers, which will be discussed later.

Polymeric particulate systems in vaccine delivery

The majority of polymeric vaccine delivery systems are based on particulate systems, consisting of microparticles, nanoparticles, polymeric liposomes, and enteric-coated particles. Particulate delivery systems present multiple copies of antigens to the immune system, and promote trapping and retention of antigens in local lymph nodes. Moreover, particles are taken up by macrophages and dendritic cells, leading to enhanced antigen penetration and the release of cytokines, to promote the induction of an immune response (O'Hagan *et al.*, 1998).

Antigens may be either surface-bound or matrix-bound within polymer particles by altering the formulation processes. Both surface-absorbed and matrix-entrapped antigens have been shown to benefit from the adjuvant effect (Li Wan Po *et al.*, 1995). However, it has been shown that the surface-bound antigens are superior in terms of inducing the mucosal IgA response (Aramaki *et al.*, 1994). In fact, the concept of using polymeric systems in vaccine delivery had been tested first in 1979 (Preis and Langer, 1979) with bovine serum albumin (BSA) encapsulated in a non-biodegradable ethylene vinyl acetate (EVAc) co-polymer, and this elicited an antibody response comparable to that seen with two injections of BSA emulsified in complete Freund's adjuvant (CFA).

Although non-biodegradable polymeric vaccine formulations are effective, there is a serious disadvantage in that a device needs retrieval after vaccine depletion. Biodegradable polymers have thus received most attention.

A polymer which is going to be used in the microencapsulation of vaccines must be biodegradable, biocompatible (i.e. non-irritant and non-allergenic), inert with respect to induction of immune responses, stable, safe (i.e. non-toxic), and easy to manufacture (Singh and O'Hagan, 1998). Polymers used in particulate vaccine delivery can be categorized as natural or synthetic polymers. 'Natural polymers' considered have been various carbohydrate polymers, such as starch (Heritage *et al.*, 1996), alginate (Bowersock *et al.*, 1999), and chitosan, as well as proteins, such as albumin and gelatin (Truong-Le *et al.*, 1998). Synthetic polymers that have been considered for use in this area include polyesters, such as poly(L-lactide) (PLA) and poly(D,L-lactide-co-glycolide) (PLG) (reviewed by O'Hagan *et al.*, 1998), polylactide-co-poly(ethylene glycol) (PELA) (Zhou *et al.*, 2003), poly(epsilon-caprolactone) (PEC) (Murillo *et al.*, 2002a,b), polymethylmethacrylate (Kreuter and Speiser, 1976), polycaprolactones (Jameela *et al.*, 1997), polyanhydrides (Chiba *et al.*, 1997), polyorthoesters, polyphosphazenes (Andrianov and Payne, 1998), imminocarbonate polymers (Kohn *et al.*, 1986), and thermally condensed amino acids (Santiago *et al.*, 1993) and ethylene-vinyl acetate polymers (Preis and Langer, 1979; Niemi *et al.*, 1985). The advantage of natural polymers is their low cost, biocompatibility and aqueous solubility, and that they do not require the harsh conditions of heat and/or organic solvents for encapsulation of antigens, as do some synthetic polymers. However, the use of natural polymers is limited due to the presence of extraneous contaminants, batch variability, and low hydrophobicity. By contrast, synthetic polymers are more reproducible, and can be prepared with the desired degradation rates, molecular weights, and co-polymer composition. Synthetic polymers are usually thymus-independent antigens, with only a limited ability to elicit antibody formation or to induce a cellular immune response against them. However, there are many other ways in which they can be used to influence the host immune system. Water-soluble synthetic polymers sometimes exhibit significant immunomodulatory activity, mainly concerning the activation/suppression of NK cells, LAK cells, and macrophages. In addition, these polymers can be tailored to meet the specific physical, chemical, and immunogenic requirements of a particular antigen, and some of them can also act as adjuvants (Rihova, 2002).

The most widely used biodegradable polymers in antigen delivery studies are the aliphatic polyesters, poly(lactide-co-glycolide) co-polymers (PLGs). The reasons that they have been considered a primary candidate include their approval by FDA for several therapeutic products because of their excellent biodegradability, biocompatibility, and a long history of safe use in humans, the capability of being formulated to release macromolecules over a long period of time, and their ability to provide pulsed-release kinetics of antigens, i.e. an initial burst, followed by a trickle release, which is favourable for current vaccination programmes (Sah *et al.*, 1995; Sanchez *et al.*, 1996). The main limitation of PLGs in relation to vaccine delivery is that these polymers are soluble only in a limited range of organic solvents, and are insoluble in water. This, in turn, results in the possibility of antigen denaturation as a consequence of exposure to organic solvents. In addition, during microencapsulation, vaccine antigens may also be exposed to high shear stress, aqueous–organic interfaces, and elevated temperature. In addition to instability during the microparticle preparation, the entrapped vaccines/antigens suffer from instability during storage of microparticles, during hydration of microparticles *in*

vivo, and during the extended periods in the body at 37°C, as reviewed by O'Hagan and co-workers (O'Hagan *et al.*, 1998).

The most commonly used method for the preparation of particulate systems with entrapped antigens involves the use of aqueous solutions of antigen, which are dispersed in an organic solvent containing the dissolved polymer. Various alternative approaches have also been described, including spray drying, phase separation (O'Hagan *et al.*, 1998), ionotropic gelation, emulsion coacervation, thermal condensation, coating on non-pareil seeds, granulation, phase inversion, and solid-in-oil-in-water (s/o/w) dispersion (Singh and O'Hagan, 1998).

Among all the candidates for particulate vaccine delivery, liposomes have a number of potential advantages. They have tremendous flexibility for incorporating hydrophilic, as well as hydrophobic materials under mild conditions, which minimizes the risk of vaccine denaturation during the encapsulation process. Liposomes are naturally taken up by macrophages. Intracellular breakdown of liposomes results in release and processing of the incorporated materials. In this way, liposomes act as immunoadjuvants, and facilitate the immune response to encapsulated vaccines (Alving, 1987; Childers and Michalek, 1994).

The susceptibility of conventional liposomes to bile salt dissolution and enzymatic degradation in the gastrointestinal tract, however, has remained as the main barrier to vaccine delivery. Disruption of liposomal membranes leads to exposure of encapsulated vaccines, resulting in the loss of their protective function. As a possible solution, polymerized liposomes have been suggested as potential carriers for vaccination. By creating a cross-linked network in the liposomal membranes, the vehicles can be stabilized both *in vitro* and *in vivo*, while being capable of being degraded intracellularly (Chen *et al.*, 1996). Liposomes have been shown, in addition, to facilitate transport of the antigens across the nasal membranes to the APC (Li Wan Po *et al.*, 1995).

Regardless of the type of particulate system and the route of administration, there are some physicochemical factors affecting the vaccine delivery efficiency of particulate systems, the most important being particle size, lipophilicity, and particle charge (Li Wan Po *et al.*, 1995). The most important in this respect is particle size, which has a significant impact on the onset and intensity of antibody generation. In general, microparticles smaller than 10 μm are favoured in terms of promoting antigen uptake by mucosal associated lymphoid tissues (MALTs), leading to enhanced antigen processing and presentation after antigen-loaded APCs migrate to the local draining lymph nodes. In contrast, formulations prepared with particles of larger sizes are generally designed to protect the antigen against degradation *in vitro* and *in vivo* (Li Wan Po *et al.*, 1995). It has been shown that increasing the hydrophobicity of the polymeric particles leads to an increased adjuvant effect with respect to antibody response (Kreuter *et al.*, 1988), as well as phargocytosis (Murillo *et al.*, 2002a).

Multimodal delivery systems which mimic single and booster doses following a single administration can be designed by altering process variables, such as polymer coat thickness, polymer type or polymeric blend composition, particle size, and particle size distribution. Combinations of particles with different particle sizes have resulted in the most promising results in terms of producing ideal pulsatile antigen release (Li Wan Po *et al.*, 1995).

Study survey

Several studies have been published in the past two decades on the development, as well as *in vitro* and *in vivo* evaluation, of polymeric vaccine delivery systems. A summary of the most recent representative studies published in this area has been listed in *Table 6.1*. It should be noted that in all the cases the controls used for observations have been the soluble and/or alum-stimulated vaccines.

POLYMERS FOR OLIGONUCLEOTIDES DELIVERY

Since the late 1970s, the application of oligonucleotides (ONs) for gene therapy of most genetic-based diseases has been well considered. ONs are single-stranded chains of nucleic acids which are able to hybridize with target nucleic acid sequences to inhibit specific proteins, and therefore allow selective treatment of various genetic, neoplastic, and infectious diseases. The administration of ONs is limited, due to their instability in biological tissue and the difficulty in delivery to the intracellular compartments of the cell (Schreier, 1994). Chemical manipulation approaches have been applied to address the instability issue, and delivery systems have been developed to increase cellular uptake of ONs. It is generally considered that ONs with or without a delivery system are transported into cells by endocytosis, and then accumulate within endosomes, where they are mainly inactivated. The rate and extent of movement of ONs from endosomes appears to be important in determining the effects of ONs. Consequently, developing accessory compounds or delivery methods that enhance endosome to cytoplasm transfer may be vital to oligonucleotide (ON) drug delivery systems (Merdan *et al.*, 2002). There are so far two different approaches to deliver ONs in gene therapy, namely viral and non-viral delivery systems. Viral vectors including retroviruses and adenoviruses are known to be highly efficient in introducing the ONs into the host cells; however, they have a couple of drawbacks, such as high immunogenicity, oncogenicity, and difficult and expensive preparation conditions. Therefore, non-viral vectors including cationic lipids and polymers are getting more attention due to their lower safety risks and ability to carry large DNA molecules. Moreover, non-viral vectors are able to condense large amounts of ONs, and are easy and cheap to prepare. The only disadvantage is their low transfection efficiency. The focus of this section is on various polymers applied as non-viral vectors for the delivery of ONs.

The polymers applied as non-viral vectors are cationic derivatives of different polymers in order to be able to condense ONs efficiently. These polymers are categorized into various groups, including mainly polyethyleneimines, chitosans, poly(lactide-co-glycolide), cyclodextrins, and dendrimers.

Polyethyleneimines

The most conventional polymers for ON delivery are polyethyleneimine (PEI) and its derivatives or complexes. Every third atom of PEI is a protonable amino nitrogen atom, which makes the polymeric network an effective 'proton sponge' at virtually any pH. Luciferase reporter genes transfer with this polycation into a variety of cell lines, and primary cells gave results comparable to, or even better than any other

transfecting vectors. The optimal PEI cation/ONs anion balance for *in vitro* transfection is only slightly on the cationic side, which is advantageous for *in vivo* delivery (Boussif *et al.*, 1995; Kunath *et al.*, 2002).

A novel series of polymers which are based on dispersed networks of cross-linked ionic and non-ionic hydrophilic polymers are the nanosized cationic network of cross-linked poly(ethylene oxide) (PEO) and polyethyleneimine (PEI), PEO-cl-PEI nanogels. Interaction of anionic amphiphilic molecules or ONs with PEO-cl-PEI results in the formation of nanocomposite materials in which the hydrophobic regions from polyion-complexes are joined by the hydrophilic PEO chains. These systems allow for immobilization of negatively charged ONs. Efficient cellular uptake and intracellular release of ONs immobilized in PEO-cl-PEI nanogel have shown the potential of enhancing oral and brain bioavailability of ONs (Vinogradov *et al.*, 2002).

Chitosans

Chitosans are linear aminopolysaccharides (considered above) for protein and peptide delivery, which have shown a significantly better biocompatibility than PEI. Chitosans are able to form small, stable and toroidal complexes with ONs. The kinetics of gene expression using chitosans is slower than that for PEI, probably due to the fact that PEI displays buffering capacity, resulting in rapid escape, whereas in the case of chitosans, polymers should be degraded within the cells, which takes longer (Erbacher *et al.*, 1998; Koping-Hoggard *et al.*, 2001).

Various chitosan derivatives are also used for ON delivery, such as N-trimethyl chitosan; however, the efficient transfection of cells using these derivatives needs to be studied in more detail (Thanou *et al.*, 2002).

Poly(lactide-co-glycolide)

Biodegradable poly(D,L lactic-co-glycolic acid) (PLGA) can be adhered to ONs to form an amphiphilic structure which is similar to an A–B type block co-polymer. A terminal end of PLGA is activated and reacted with primary amine-terminated ONs. This process can be via a conventional encapsulation method. The encapsulation efficiency can be increased with decreasing internal water content, decreasing stirring time prior to filtration of conjugates and decreasing ONs loading (Freytag *et al.*, 2000). The ONs/PLGA conjugates self-assemble in aqueous solution to form a micellar structure by serving PLGA segments as a hydrophobic core, and ONs segments as a surrounding hydrophilic corona. These micelles are able to release ONs in a slow manner by controlled degradation of hydrophobic PLGA chains. Compared to unconjugated ONs, the ONs/PLGA micelles appear to be more efficiently transported within cells, presumably by endocytosis (Jeong and Park, 2001). These biodegradable PLGA polymers have great potential as an efficient delivery system for ONs in HIV natural target cells (Berton *et al.*, 2001), tumour cells (Delie *et al.*, 2001), and inhibition of glioblastoma cell line growth (Gill *et al.*, 2002).

It is also possible to incorporate ONs and PEI complexes at nitrogen to phosphate (N/P) molar ratios of about 15 or 40 into poly(lactide-co-glycolide) microspheres by the multiple emulsion-solvent evaporation technique. ON/PEI

Table 6.1. Polymeric delivery systems used for vaccine delivery

Antigen	Delivery system	Polymer	Route	Observation (ref.)
HIV vaccines	Nanoparticles	Poly methyl-methacrylate	Parenteral	Higher antibody titres (Stieneker *et al.*, 1991)
Influenza virus antigens	Microparticles	Poly(amino acids)	PO[1]	Higher anti-haemagglutinin and neuramidase acid responses (Santiago *et al.*, 1993)
Ovalbumin (OVA), extracts of *Pasteurella multocida*, flagellin of *Salmonella enteritidis*	Microspherical hydrogels	Sodium alginate	PO	Production of sIgA at the mucosal surfaces and increased delayed-type hypersensitivity (Bowersock *et al.*, 1999)
Small synthetic peptides representing measles virus cytotoxic T lymphocyte epitopes	Microparticles	PLG[3]	PO	Priming of splenic peptide-specific cytotoxic T lymphocyte (CTL) responses (Partidos *et al.*, 1999)
BSA[2]	Microparticles	PEC[4]		Entrapped protein seemed to remain unaltered by the protein encapsulation process with a BSA loading and entrapment efficiency reaching, respectively, 5% and 30% (Benoit *et al.*, 1999).
Tetanus toxoid (TT)	Microcapsules	PLG		Over 92% of the TT released over a 63-day period (Sanchez *et al.*, 1996)
A hetero species dimer of alpha-ovine luteinizing hormone and beta-human chorionic gonadotrophin linked to diphtheria toxoid	Microspheres	PLG	IM[5]	A single injection of immunogen entrapped in the microspheres generated a birth control response comparable to that obtained by the same immunogen on alum injected at a monthly interval (Singh *et al.*, 1995)
A synthetic peptide analogue of hepatitis B surface antigen	Microspheres	Oligo-saccharide ester derivatives (OEDs) of trehalose	Parenteral	High titre anti-hepatitis B (anti-HBs) surface antigen antibodies (Moynihan *et al.*, 2002)
HBsAg and beta-galactosidase	Nanoparticles		IN[6]	High levels of specific serum IgG antibodies and cytotoxic T lymphocyte (CTL) responses. Moreover, specific IgA antibodies were found in nasal as well as in vaginal washes (Debin *et al.*, 2002)

An antigenic complex from *Brucella ovis*	Microparticles	PLG, PEC		The release profile for PLGA microparticles was continuous, whereas PEC ones released the antigens in a triphasic release pattern and they are greatly phagocytosed by macrophages (Murillo *et al.*, 2002a).
Diphtheria toxoid (DT)	Microparticles	Chitosan	PO, IN	Protective systemic and local immune response against DT after oral vaccination, and in significant enhancement of IgG production after nasal administration (van der Lubben *et al.*, 2003)
Two adjuvants, cholera toxin (CT) and lipid A (LA), with plasmid DNA (pDNA)	pDNA-coated nanoparticles		SC[7], topical	Enhanced the antigen-specific serum IgG titre by 4- and 20-fold by the topical route; pDNA-coated nanoparticles adjuvanted with 10 μg CT resulted in the strongest splenocyte proliferation by the SC route (Cui and Mumper, 2003).
Streptococcal glucosyltransferase	Microparticles	PLG	Rectal	Saliva and nasal washes of all intranasally immunized rats contained IgA antibody to glucosyltransferase on day 28 (Smith *et al.*, 2003).
Live porcine rotavirus (PRV) or its recombinant VP6 protein	Microspheres	Alginate	IP[8], PO, enteric	VP6-specific IgG (but no IgA) antibodies were detected in the sera of mice after a single IP immunization; oral immunization with VP6-MS induced the highest level of VP6-specific faecal IgA antibody (Kim *et al.*, 2002).
pDNA	pDNA-coated nanoparticles	PLG	IN	Enhanced serum IgG and IgA titres to an expressed model antigen, beta-galactosidase, by 18–28- and 25–30-fold, respectively; an enhanced splenocyte proliferative response was also observed (Cui and Mumper, 2002).
Vibrio cholerae (VC)	Microparticles	PLG	PO	Vibrio-specific serum IgG and IgM responses as well as vibriocidal antibody activity in mice (Yeh *et al.*, 2002)
Antigenic extract Hot Saline from *Brucella ovis*	Microparticles	PLG, PEC		All formulations showed light toxicity by the MTT[9] assay; particles prepared with PEC showed the higher uptake by J744-macrophages and cell respiratory burst (Murillo *et al.*, 2002a)
A branched HIV-1 principal neuralizing determinant (PND)	Microparticles	PLG	PO, SC	High levels of both IgG and neutralizing antibodies against HIV (O'Hagan *et al.*, 1995)

[1]per oral, [2]bovine serum albumin, [3]poly(lactide-co-glycolide), [4]poly ε-caprolactone, [5]intramuscular, [6]intranasal, [7]sub-cutaneous, [8]intraperitoneal, [9]MTT is a colorimetric microtitre and measures the ability of viable cells to reduce a tetrazolium salt.

complexes encapsulated inside microspheres are protected against enzymatic degradation in fetal calf serum. Interestingly, ON/PEI complexes slowly released from microspheres efficiently penetrated inside HeLa cells and ONs are able to be located in the nucleus (de Rosa *et al.*, 2002).

Cyclodextrins

ONs can be transferred into tumours using cationic liposomes and cyclodextrins (CyDs). Cationic liposomes are generally 100–200 nm in diameter, whereas CyDs typically span 1.5 nm across. CyD molecules are routinely used as agents that engender cholesterol efflux from lipid-laden cells, thus having an efficacious potential in the management of atherosclerosis by introducing ON molecules into the nucleus of the cells (Dass, 2002). The peculiar properties of CyDs could be exploited in such an emerging therapeutic area by virtue of their capability of interacting with cellular membranes, thus giving rise to improved cellular uptake of ONs (Redenti *et al.*, 2001).

Dendrimers

Dendrimers are highly branched macromolecules synthesized by multiplication of a series of repetitive units, typically polyamides. Dendrimers are cationic polymers that have been used for the delivery of ONs to the cells. However, little is known about the behaviour of dendrimer–nucleic acid complexes once they reach the cell interior. The dendrimer–ONs complex remains associated during the process of uptake into vesicular compartments, and eventual entry into the nucleus. Since the pharmacological activity of the antisense compound is manifest under these conditions, it suggests that the dendrimer–ONs complex is functionally active (Helin *et al.*, 1999; Yoo and Juliano, 2000). Moreover, dendrimer–ONs remain intact intracellularly and, although they probably bound to the plasma membrane, and especially in endosomal compartments, also co-localized in the cytoplasm and nucleus to a much higher value than free ONs (Bielinska *et al.*, 1996; Henke *et al.*, 2000).

Conclusions

Pharmaceutical and biopharmaceutical applications of polymers and polymeric systems are widespread, due to the variety of their structures and functionality. In this review, we have considered the two main categories of polymers – biodegradable and non-biodegradable – and their applications in drug delivery. It was considered that biodegradable polymers have more advantages than non-biodegradable polymers for drug delivery; however, non-biodegradable polymers still offer numerous benefits for controlled release of drugs. In the second part of this review, various polymers, which are used in the delivery of biopharmaceuticals, including peptides, proteins, genes, vaccines, and oligonucleotides, were scrutinized. It is clear that there are a large number of biodegradable polymers which have the potential to be applied in the delivery of biopharmaceutical compounds via various routes of administration.

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